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PAPER 8A/5

LEGUME VIRUS RESEARCH IN THE NETHERLANDS WITH SPECIAL REFERENCE TO FORAGE CROPS

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Summary. Of 10 or more different legume viruses found in The Netherlands, this paper surveys work on 4, viz. the red clover vein-mosaic virus, the top yellows virus of peas in lucerne, the white clover mosaic virus and the witches' broom virus of white clover.
The control of virus diseases in legumes is briefly discussed.
Many legume viruses are widely distributed, and the importance of international co-operation in legume-virus research is stressed, particularly in devising internationally accepted procedures for virus identification.

Résumé. Cette communication concerne le travail effectué sur 4 virus parmi la grosse dizaine de virus différents des légumineuses trouvés aux Pays-Bas. Ce sont: le virus de la mosaïque nervaire du trèfle des prés, le virus de la jaunisse du pois chez la luzerne, la mosaïque et le virus du balai de sorcière du trèfle blanc.
La lutte contre les maladies à virus des légumineuses est brièvement commentée.
De nombreux virus des légumineuses sont largement disséminés et l'accent est mis sur l'importance de la coopération internationale dans le domaine de la recherche sur les virus des légumineuses, particulièrement dans la mise au point de procédés internationalement admis pour l'identification de virus.

Zusammenfassung. Von 10 oder mehr verschiedenen Leguminosenviren, welche in den Niederlanden bekannt sind, werden in dieser Arbeit vier auf Futtergewächsen vorkommenden Viren erwähnt, nämlich das Rotkleeadernmosaikvirus, das Blattrollvirus der Erbse in Luzerne, das Weisskleemosaikvirus und das Hexenbesenvirus des Weissklees.
Die Bekämpfung von Viruskrankheiten in Leguminosen wird kurz besprochen.

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Viele Leguminosenviren sind weit verbreitet und deshalb wird die Bedeutung internationaler Zusammenarbeit in der Leguminosenvirusforschung, besonders mit Bezug auf die Entwicklung von international anerkannten Verfahren zur Virusidentifizierung, betont.

Introduction

In the Netherlands legumes are of special importance in arable farming and to a lesser extent in grasslands. The first Dutch publication, dealing with some preliminary research on legume viruses, appeared in 1928 (12). After World War II research was intensified, especially on beans and peas (9, 10, 13), the most important leguminous crops in our country. Lists of publications up to and including 1958 have been published (5, 14).

From these studies and those in other countries it soon appeared that clovers and other perennial leguminous plants occurring in grasslands or along roadsides and on waste ground, commonly harbour viruses of annual pulse crops. Other facts are that, in legumes, multiple infections often occur and that different viruses may induce similar symptoms.

In 1957 the authors started to study more systematically the occurrence of legume viruses in the Netherlands, treating them as one group. This paper describes briefly this research on viruses of forage legumes and some of the results.

Scope of the investigations

In making a survey of the legume viruses occurring in our country, difficulty was found in identifying the viruses by reference to the international literature. Therefore the viruses isolated in the survey were studied in detail to get as much information on them as possible. In addition to host range, symptomatology (symptoms varied considerably with change of environment) and mode of transmission, the intrinsic characters of the viruses were especially studied, namely, physico-chemical properties, serological behaviour, and size and shape as revealed by electron-microscopy. Only in this way could the viruses concerned be identified, and the future diagnosis of the diseases be facilitated.

Table 1 gives a summary of the legume viruses detected so far in the Netherlands, together with their natural hosts. Because more plant species can be infected by experimental inoculation than are found naturally infected it is presumed that most viruses have a broader natural host range than is indicated in this Table. The Table clearly demonstrates that any one virus can infect several different legume crop species and suggests that clovers are important in the overwintering of viruses of pulse crops. In this Table, pea mosaic virus and bean yellow mosaic virus are mentioned separately. In the literature and from our own experiments, however, data are available suggesting a close relationship (perhaps a strain relationship) between the two. The same holds true for lupin mosaic virus, for in 1958 Corbett (6) demonstrated that, in Florida, lupin mosaic is caused by bean yellow mosaic virus.

Some results of the investigations

RED CLOVER VEIN-MOSAIC VIRUS, which causes a conspicuous vein mosaic in red clover, and symptoms in white clover and some other legumes, also causes pea stunt. Both in red clover and in peas the disease was observed for the first time in our country in 1957. In our

TABLE 1

SURVEY OF VIRUSES OF LEGUMES OCCURRING IN THE NETHERLANDS

Host plants	Pea	Bean	Scarlet runner	Broad bean	Red clover	White clover	Hybrid clover	Crimson clover	Lucerne	Yellow lupin	Blue lupin
Viruses											
Bean yellow mosaic	+	+	—	+			+	+		+	+
Common bean mosaic		+	—	—							
Lupin mosaic										+	
Pea enation mosaic	+			+			+			+	
Pea top yellows (leaf roll)	+			+					+		
Pea mosaic	+	—	—	+	+		+	+			+
Red clover vein mosaic	+	—	—		+	+	+	+			
White clover mosaic	+				+	+					
Lucerne mosaic	+	+			+	+		+	+		
Bean stipple streak		+	—								
Cucumber mosaic	+						+			+	+
Witches' broom of white clover						+					

+ = natural infection established in our own experiments or according to literature.
— = non-susceptible after experimental inoculation.

opinion this virus occurs only sporadically. The virus has been studied in detail (8); an antiserum was prepared and electron-micrographs made. The virus probably has a world-wide distribution.

Top yellows virus of pea (pea leaf roll virus) in the Netherlands occurs principally in regions where much lucerne is grown. The virus vector, the pea aphid (*Acyrthosiphon pisum*) overwinters in lucerne fields (7). Since 1950 a brilliant vein yellowing has been observed in this crop, variety Du Puits, and insect-transmission tests show that this symptom is caused by infection with top yellows virus of pea (15). The vein yellowing is usually entirely masked during the summer. Apart from this symptom, no other abnormalities could be observed. Because the virus does not kill lucerne plants, infected plants act as sources of this virus for several years. As yet there are no data to show whether the top yellows virus affects the yield and quality of the lucerne crop.

White clover mosaic virus has been isolated several times not only from white clover but also from peas and red clover. Because infection is often masked in white clover and because symptoms in red clover and in peas are often not diagnostic, infection is difficult to recognize in the field. Therefore it is unknown to what extent this virus occurs and what its economic importance is. In view of its extremely high infectivity and the ease of transmission by contact it seems likely to be widespread. So far the disease has been reported only in the USA, the Netherlands and Germany, but it probably has a much wider distribution. The results of our detailed studies (3) indicate that it is related to, even if not identical with, the American white clover mosaic virus described in the literature. However, Johnson (11) claimed that white clover mosaic in the USA is caused by a double infection with pea mottle and pea wilt viruses. We could obtain no evidence that white clover mosaic in the Netherlands is caused by such a double infection. White clover mosaic virus is also able to infect a number of non-leguminous plants, e.g. cucumber. As has just been mentioned, in white clover the virus is often carried symptomlessly. Whether or not infected clover plants yield less than healthy plants, and whether or not their resistance to frost is impaired, have still to be investigated.

Witches' broom virus of white clover is the last interesting and important virus to be mentioned here. Research on this is still going on and only some preliminary results will be mentioned here (cf. also (2)). On the basis of our own observations, the literature and personal communications from several colleagues in Europe, witches' broom occurs not only in the Netherlands, but also in Germany, Switzerland, Italy, Yugoslavia, Hungary, Czechoslovakia, Poland, England and Ireland. In our country and in Germany the disease occurs occasionally, usually in a few plants only. However, under certain conditions the disease may become epidemic: it did so twice recently in breeding stations in our country, leading to the complete destruction of white clover clone collections. The disease may be of economic importance in clover in warmer countries like Italy and Czechoslovakia. Diseased plants become sterile. In those countries where it occurs epidemically the disease may be very destructive.

The exact identity of the causal virus and its relationship to other 'witches' broom viruses' has not yet been established. These viruses include aster yellows in the USA, potato witches' broom in Czechoslovakia, tomato big bud in Australia, green petal of strawberry in England and presumably 'stolbur' of potato in central and south-eastern European countries, and all these may induce similar witches'-broom type symptoms in white clover. In addition to witches' broom growth the symptoms in white clover include antholysis, virescence, phyllody and prolification of the flowers. Antholysis leads to complete sterility. There is also some vein clearing and yellowing of the leaves. No witches' broom viruses are transmissible by inoculation of sap, but they are leafhopper-borne. The insects become infective from 1 to several weeks after they acquire the viruses from diseased plants and can infect for the rest of their lifetime. So the epidemiology of these diseases depends upon the spread of the leafhoppers, which in its turn is governed by the climate. The witches' broom viruses have a wide host range, and white clover may be important as a reservoir of infection for other crops. There is much about these diseases which is not known, and they deserve careful attention.

Control of virus diseases of legumes

Because virus diseases cannot generally be cured, their control lies in preventing infection, which requires detailed knowledge of their epidemiology. Commonly, legume viruses have many winter host plants, namely perennial crops and plants occurring in meadows. Therefore it is difficult to prevent plants in the neighbourhood of possible virus sources from becoming infected. Control of virus-transmitting insects is also difficult, because to prevent virus spread the insects should be killed before they move from perennial to annual crops. Often, as for example with lucerne infected with top yellows virus of pea, the virus is not harmful to the plant and the farmer is neither interested in spraying the lucerne nor is he concerned with the danger of exposing his neighbour's peas to infection with the top yellows virus. Moreover, particularly in the Netherlands, the plots are relatively small and consequently it is hardly possible to apply insecticides in a practical, economic way. These facts mean that the only way to prevent damage by legume viruses is to use resistant varieties, and there are, as yet, few of these. To breed resistant varieties, knowledge of the diseases and of the viruses involved is a prerequisite. With peas and beans, which are self-pollinated, varieties resistant to some viruses, e.g. top yellows of peas, have been produced. In

clovers, which are cross-pollinated, the situation is complex and breeding for resistance to viruses will be difficult. Growing clover crops for a restricted period — e.g. not longer than 2 years — in an arable crop rotation might be advisable as a mode of preventing the build-up of virus infection to high levels.

International co-operation in legume virus research

As has been pointed out, many legume viruses occur in different regions of the world, and international co-operation in research on viruses of leguminous crops is very important. As an aid to making results obtained in different countries easier to compare, a report entitled 'Suggested procedures for international legume virus identification' (4) has been prepared in collaboration with Professor D. J. Hagedorn, Madison, Wisconsin, USA, and Dr L. Quantz, Braunschweig, Germany. This report has been sent to colleagues in various countries, together with a letter suggesting the establishment of an International Working Group on Legume Viruses. One of the aims is to exchange seeds of host plants, antisera, data obtained by electron microscopy, etc. It is hoped, in this way, to partition problems, and favourable replies have been received.

Discussion

In the Netherlands, in contrast with other parts of the world, legumes are of little importance in permanent grasslands. Because nitrogenous fertilizers are used, clovers are gradually disappearing from our grasslands. In ley-farming, red and white clover are often included in the seeds mixture. How far viruses contribute to the disappearance of clovers from meadows is not yet known but in our opinion their importance in this connection is small.

In our country, legumes are grown as forage crops and sometimes as soil cover crops. Viruses are then important, especially in lucerne. This crop usually lasts for more than 2 years, and particularly in the south-western part of the country conditions favour the spread of pea top yellows virus, resulting in the build-up of a high percentage of infection during the life of the crops.

In the Netherlands legumes in forage crops and grasslands are important as overwintering hosts of viruses which can be spread to annual legumes that are often sensitive to infection. Damage done to clovers and lucerne is usually not obvious but needs further careful research.

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